bio-protocol Preprint

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Whole mount immunostaining

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Updated date: Apr 13, 2020

An abbreviated version of this protocol was published in eLIFE in May 2019

Identification of EOMES-expressing spermatogonial stem cells and their regulation by PLZF

DOI: 10.7554/eLife.43352

Detailed protocol

Whole mount staining protocol for mouse seminiferous tubules.

The seminiferous tubules were teased apart in PBS using 5-Number forceps.

The tubules were washed with PBS 3times and fixed in 4% PFA in PBS at 4oC for 3-4hrs if staining for GFRA1. (Time varies with antibodies used)

After 4hrs the tubules were washed in PBS 3 times for 10 min each (no permeabilization needed for GFRA1 antibody). (Might require permeabilization for nuclear proteins, 15min incubation in 0.1% Triton-X 100)

The tubules were then incubated in 5% BSA (Sigma # A9647) in PBS (no detergent) for 1hr at RT.

The tubules were incubated with Anti-GFRA1 antibody (1:250) in 5% BSA-PBS overnight at RT (the GFRA1 antibody works better at RT than 4oC).

Next day the tubules were washed 3 times for 10 min each in 0.1% Triton-X 100, 1% BSA in PBS.

The tubules were then incubated with secondary antibody (1:1000) conjugated to Alexa- fluorophore for 1 hr at RT.

Tubules were again washed in 0.1% Triton-X100 in PBS 3times for 10min each.

Tubules were then mounted in mounting media from Vectashield without DAPI and imaged on Confocal microscope.

Related files

Wholemount staining protocol.docx

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sharma, M. (2020). Whole mount immunostaining. Bio-protocol Preprint. bio-protocol.org/prep273.
2. Sharma, M., Srivastava, A., Fairfield, H. E., Bergstrom, D., Flynn, W. F. and Braun, R. E.(2019). Identification of EOMES-expressing spermatogonial stem cells and their regulation by PLZF. eLIFE. DOI: 10.7554/eLife.43352